anti-apoptotic mediator in cancer cells. Furthermore, ADI causes arginine depletion enzymatically, and thus ADI is an inhibitor of NO synthesis by arginine substrate deprivation. However, the mechanisms of NO in ADI treated cell lines have not been previously elucidated. Here, we analyzed the mechanisms of NO in an ADI treated cell line.
We selected the Ramos human lymphoma cell line, a known ADI sensitive cell line. Having determined the optimum ADI concentration for experimentation, the cells were divided into several groups based on SNP (an NO donor) treatment levels (i.e., a ADI and SNP untreated control, ADI without SNP, ADI with 10 μM/ml SNP, ADI with 50 μM/ml SNP, and ADI with 100 μM/ml SNP). The MTT assay was used to determine cell survival fractions, nitric oxide assays to determine NO levels, and Western blot analysis to determine the expressions of NO mediators, such as NFκB and *Bcl-X_L* antibody.
The optimal ADI experimental concentration was 0.001 U/mL. Surprisingly, SNP treatment reversed ADI induced cell growth inhibition. Furthermore, we found that NFκB and *Bcl-X_L* expressions were induced by SNP.
We believe that ADI-induced Ramos cell growth inhibition is reversed by the NO donor SNP, and that this is mediated by NFκB and *Bcl-X_L*.

217 PUBLICATION

Phase I of intermittent chronomodulated oral therapy with capecitabine in patients with advanced and/or metastatic cancer

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Background: Capecitabine is an orally administered pro-drug of 5-fluorouracil (5-FU) which goes through the intestinal mucosal membrane as an intact molecule. It is subsequently activated by a cascade of three enzymes resulting in the preferential release of 5-FU at the tumor site. In phase I study capecitabine was administered twice daily as outpatient therapy, each cycle administered for 2 weeks followed by 1 week of rest. The recommended phase II dose was 2510 mg/m^2 daily. The rational of capecitabine administration especially in nocturnal hours, as performed in the present report, is just based on the attempt to mime 5-FU chronomodulated infusion.
Material and Methods: The aim of this study was to determine the maximum tolerated dose (MTD) of capecitabine when administered in a chronomodulated way according to the following schedule: 1/4 dose at 8:00 a.m.; 1/4 dose at 6:00 p.m. and 1/2 dose at 11:00 p.m. each day for 14 consecutive days followed by 1 week of rest. A total of 24 pts (17 female, 7 male), aged 49–88 yr (median 75) with a variety of solid tumors (11 breast, 7 colorectal, 2 pancreas, 1 gastric, 1 renal, 1 hemangiopericytoma and 1 unknown primary) have been treated. The starting dose level in our phase I trial was 1500 mg/mq daily. The subsequent dose levels were: 1750, 2000, 2250, 2500, 2750 mg/m^2 daily. The capecitabine dose was escalated when almost 3 patients in a cohort had completed two cycles of treatment. Dose-limiting toxicities (DLT) were determined on the basis of toxicity from the first two cycles. The MTD was defined as the dose level at which no more than one of six patients experienced a DLT. The MTD represents the dose recommended for further studies. All patients except five had been pretreated for cancer.
Results: No DLT occurred at doses of 1500, 1750, 2000, 2250 and 2500 mg/m^2 in any of the almost 3 patients included at each level. At 2750 mg/m^2, 1 of 6 patients experienced DLT (fatigue grade 4 and diarrhoea grade 3). Another 6 patients are being evaluated at the capecitabine 2750 mg/m^2 level, with an ongoing evaluation of cumulative (all cycles) toxicity and efficacy. The other toxicities have been generally mild or moderate in nature with only one case of severe hand–foot syndrome being observed at the fourth cycle in one patient. All these toxicities resolved upon treatment interruption with patients restarting on the chronomodulated schedule where appropriate. The recommended dose for further studies is 2750 mg/m^2 daily for 14 consecutive days followed by 1 week of rest. In terms of response, we have observed 5 PR in breast cancer and 2 PR in colorectal cancer.
Conclusions: In conclusion, chronomodulated capecitabine treatment seems to be a feasible approach which has demonstrated promising clinical activity.

218 PUBLICATION

The investigation of phosphatidylinositol 3-kinase (PI3k) isoforms which express by human prostate cancer cell lines, PC-3 and DU-145

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Matrix metalloproteinases (MMPs) are the most important enzymes which not only degrade basement membranes but also involve in angiogenesis and neovascularization; making possible cellular migration. Phosphatidylinositol 3-kinase (PI3K) is involved in modulating MMPs activities. PTEN is a tumor suppressor gene whose primary function is lipid phosphatase. By dephosphorylating phosphatidyl inositol 3, 4, 5-triphosphate, PTEN antagonizes the PI3K activity. Prostate cancer is one of the most prevalent cancers all over the world. Two highly invasive and metastatic cell lines from prostate cancer, PC3 and DU145, are not the same in respect to PTEN expression status. While DU145 express PTEN mRNA and its protein, PC3 is null for PTEN gene. Nevertheless PC3 is also invasive and metastatic, it was isolated from prostate cancer metastasis to the bone marrow. It was interesting for us to know if there was any difference in PI3K isoforms expression patterns between these two cell lines. For this reason the mRNA content from the cells was analyzed using RT-PCR method.
Surprisingly our data showed that both of the cell lines express identical isoforms. Here, we introduce P110α catalytic subunit and P85 adapter protein from classIA, PI3K-C2 from classII and Vps34p from classIII of PI3K super family as PI3K isoforms which expresse by PC3 and DU145 cells. Now to address any inequality in PI3K isoforms expression, using Real-Time RT-PCR we are going to quantify each isoform mRNA individually.

219 PUBLICATION

Expression and activity of signal transducer and activator of transcription (STAT) pathways in gastric adenocarcinoma

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Objective: Gastric cancer is initiated and progressed through a number of signaling pathways. In the present study, we investigated the expression and activity of signal transducers and activators of transcription (Stat) in gastric cancer cell line as well as in tissues and their relationship with clinicopathological parameters.
Methods: We have obtained 62 tissue specimens from 16 patients of surgically resected gastric adenocarcinoma and AGS gastric cancer cell line. Western blotting of gastric cancer tissues, adjacent normal tissues and AGS cell line were used to detect the expression of Stat1, Stat3 and Stat5. The expression intensity of phosphorylated STAT protein in gastric cancer tissues and adjacent normal tissues were measured by immunohistochemical stains.
Results: Of 16 patients with gastric adenocarcinoma, 14 were male and 2 were female, and median age was 66 years (range, 37 to 80). Eleven patients were at stage III or IV without distant metastasis, while 5 were at stage I or II. Activations of Stat1, Stat3, and Stat5 were observed in AGS cells, gastric cancer tissues and adjacent normal tissues. No significant difference in Stat activity was found between gastric cancer tissues and adjacent normal tissues. Furthermore, STAT activity did not correlate with stage, tumor penetration and nodal spread.
Conclusions: Expressed in gastric cancer tissues and adjacent normal tissues, Stats may play a critical role for development and adjacent penetration in gastric adenocarcinoma.

220 PUBLICATION

Bisphosphonates down-regulate the GAPDH gene expression in prostate and breast cancer cell culture: is the GAPDH a housekeeping or a new target gene?

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The employment of the RT-PCR method has been widely used for the analysis of gene expression in many systems, including tumor samples. The GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) has been commonly considered as a constitutive housekeeping gene to normalize the specif gene expression. However the GAPDH has been shown to be upregulated in cancer. Bisphosphonates (BPs), synthetic analogs of pyrophosphate, are potent inhibitors of bone resorption and recently an antitumor effect has been shown *in vitro* and in animal models by inhibition of the mevalonate pathway. Furthermore BPs has been shown to modulate many gene expression not only in osteoclasts but also in cancer cells.
The aim of this study was to evaluate GAPDH gene expression by real time RT PCR (Applied Biosystems) in different breast (MCF-7 and T-47D) and prostate cancer cell lines (PC-3 and DU-145) lines (purchased from ATTC Rockville, MD, USA). treated with amino and non-amino bisphosphonates (clodronate, pamidronate, alendronate and zoleronate) to exclude, if any,

effects of BPs on GAPD mRNA expression, and to explore the suitable of GAPDH as housekeeping gene in gene expression studies. Cells were treated for 48 h with BPs with doses of 10, 50 and 100 μM. For each concentration three experiments were performed. The housekeeping gene B2M was used to normalized GAPDH mRNA expression.
Our results show a significant dose-dependent downregulation of GAPDH gene expression after treatment of different cancer cells line with different amino-BPS. Zoledronate resulted the most powerfull bisphosphonate, whereas Clodronate, a non-amino BP, exerted significant effect on GAPDH expression only with the highest concentration tested.
In conclusion, the use of GAPDH as a control gene, in particular in studies investigating the effects of BPs on bone or cancer cells, should be inappropriate and RT PCR data on the effects of BPs in cancer cell should be reviewed, utilizing a different house keeping gene, i.e. B2M. On the other hand, this gene could be considered as a novel target gene for BPs on cancer cells.

221 PUBLICATION

Changes in the regulatory mechanism of protein synthesis induced by the combined antimigratory action of borrelidin and CGP57380

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Background: Borrelidin an inhibitor of threonyl-tRNA synthase inhibits angiogenesis, induces apoptosis and reduces tumor metastasis. Present studies addressed the questions whether cluster- or amoeboid-type of tumor cell migration are similarly affected and whether translational factors are implicated in this activity. Furthermore the modulation of the antimigratory potency of borrelidin by threonin and CGP57380 was tested.
Materials and methods: Migration of HT-1080 fibrosarcoma cells and ZR-75−1 mammary adenocarcinoma cells were studied both in 3D tissue culture, containing matrigel allowing cluster-type of migration and wound-healing assay, resulting in amoeboid cell-movements in monolayer. Western immunoblot technique was used to detect phosphorylated and non phosphorylated molecules participating in signal transduction, actin was studied by applying immunocytochemical technique.
Results: Migration of HT-1080 fibrosarcoma cells could be inhibited both in 3D cell-culture and in wound healing assay. Interestingly the antimigratory action of borrelidin was abbrogated in the wound-healing assay, by threonine which offered protection against the cell-kill action of borrelidin and also by CGP57380 an inhibitor of MNK-1. In fact both compounds enhanced the antimigratory action of borrelidin in the 3D cell-culture. Inhibitory action of borrelidin against global protein synthesis was further reduced by CGP57380 and also by inhibitors of SAPKp38 and PI3-K, however, abbrogated by threonine. Borrelidin enhanced the phosphorylation of SAPKp38 and eIF-4E, increased the expression of HSP-27, reduced the activity of MMP-9 and MMP-2 and the expression of integrin αvβ5, and in addition the cellular localization of F-actin was redistributed.
Conclusions: It may be assumed that the proapoptotic and antimigratory action of borrelidin are the consequence of dysregulated cross-talks among the translational factors, preferentially eIF-4E and HSP-27 with the subsequent alterations in the functions of the cytoskeletal system. The opposite response of the same tumor cell population, participating in cluster- or in amoeboid-type of migration to the inhibitory action of borrelidin in the presence of CGP-57380 or threonine indicates that two different molecular mechanisms are implicated in these two migratory processes.

222 PUBLICATION

Hepatic arterial injection with 5-fluorouracil and dihydropyrimidine dehydrogenase inhibitor for the metastatic liver tumor in rabbits

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Backgrounds: Hepatic metastasis is one of the most important prognostic factors of cancer in digestive organs, especially gastric and colorectal cancer. In many cases of multiple hepatic metastases, surgical resection is impossible and hepatic artery injection (HAI) with fluoropyrimidine anticancer drugs such as 5-fluorouracil (5-FU) is commonly performed using a reservoir. However, 5-FU is rapidly degraded to α-fluoro-β-alanine after contact with dihydropyrimidine dehydrogenase (DPD) which is mainly present in the liver. Recently, a novel oral fluoropyrimidine anticancer drug, S-1 was developed and contains fluorinated pyrimidine and 5-chloro-2, 4-dihydorxypyrimidine (CDHP) as a DPD inhibitor. We investigated the pharmacokinetics of HAI with 5-FU and CDHP in the experimental model.
Materials and methods: VX2 tumor cells were inoculated into the hepatic parenchyma at single site of the rabbits. Two weeks later, the rabbits were divided into two groups. Group A: 10 mg/kg of 5-FU was continuously administered into the hepatic artery for one hour. Group B: 10 mg/kg of 5-FU and 4.5 mg/kg of CDHP were continuously administered into the hepatic artery for one hour. In each groups, samples were collected from the plasma, normal liver tissue and liver tumor tissue in the same lobe, at 0, 1, 2, 4, 8, 12 hours after intra-hepatic arterial infusion. The levels of CDHP and 5-Fu in the plasma, normal liver tissue and liver tumor were measured. The levels of DPD activity, thymidylate synthase inhibition rate (TSIR) and 5-FU incorporated into the RNA fraction (F-RNA) in the normal liver and liver tumor were investigated.
Results: The level of CDHP positively correlated with that of 5-FU and negatively correlated with that of DPD activity. The levels of CDHP, 5-FU, TSIR and F-RNA in group B were higher than those in group A and the level of DPD activity in group B was lower than that in group A ($p < 0.05$). In group B, the levels of CDHP, 5-FU and TSIR in the liver tumor were higher than those in the normal liver tissue and the level of DPD activity in the liver tumor was lower than that in the normal liver tissue ($p < 0.05$).
Conclusion: HAI with 5-FU and CDHP suspected to be effective for unresectable metastatic tumors in the liver in which DPD abound.

223 PUBLICATION

Nuclear translocation of Abl tyrosine kinase in the apoptotic response to DNA damage

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The ubiquitously expressed tyrosine kinase c-abl is localized in the cytoplasm and the nucleus. Nuclear c-Abl is activated by diverse genotoxic agents and induces apoptosis mediated by p73 or hRad9, although the regulation of DNA damge-induced c-Abl activity remains unclear. Here we show that c-Abl accumulates in the nucleus in response to DNA damage. Nuclear targeting of c-Abl is independent of its kinase activity.
The results also demonstrate that 14−3-3 proteins interact with c-Abl predominantly in the cytoplasm. c-Abl phosphorylation at Thr 735 located near the nuclear localization signal (NLS) is responsible for binding to 14−3-3. The mechanism by which genotoxin exposure disrupts sequestration of c-Abl by 14−3-3 in the cytoplasm is supported by the finding that JNK phosphorylates 14−3-3 that no longer associates with c-Abl. In concert with these results, the expression of unphosphorylated mutant of 14−3-3 suppresses nuclear accumulation of c-Abl and induction of apoptosis.
Taken together, these findings demonstrate that 14−3-3 is the essential regulator for c-Abl in the intracellular localization and in the apoptotic response to DNA damage.

224 PUBLICATION

Cytotoxic and cell cycle effects induced by two aqueous-etanol herbal extracts on human cervix carcinoma and human breast cancer cell lines

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Background: In recent time there is an increasing interest for the anticancer activities of extracts of different plants. The goal of this study was to examine the *in vitro* cytotoxic effects of the aqueous-ethanol extracts, *uman*[b] and *uman*[c] provided by 17 and 12 herbs respectively, originating from Serbia and Montenegro, to two human breast cancer cell lines (MDA-MB-361, MDA-MB-453), to human cervix carcinoma HeLa cells and to normal human PBMC.
Methods: Antiproliferative action was determined indirectly, measuring target cell survival *in vitro*, by two assays: by metabolic MTT, and by Kenacid BlueR (KBR) dye binding method. Fresh extracts were used for each experiment. The effects of investigated extracts on the cell cycle was measured after PI staining by flow cytometry. The cell cycle distribution was estimated from the DNA frequency histograms of diferent cell lines after 24 h, 48 h and 72 h of the extracts action. Concentration of the investigated extracts were equal to IC50 or two IC50. Besides, the mode of cell death (apoptosis and/or necrosis) was examined by fluorescence microscopy, using acridine orange and ethidium bromide stained cells.
Results: Examined extracts exerted the antiproliferative action to neoplastic lines IC50 being less than 20μl of extract per ml of nutrition medium. The order of sensitivity of various cell lines determined by both assays was: cervix HeLa > MDA-MB-453 > MDA-MB-361, for *uman*[b], and MDA-MB-361 > MDA-MB-453 > cervix HeLa for *uman*[c]. At this range of extracts concentrations (<20 μl/ml), the extracts did not exert any significant cytotoxicity toward healthy human PBMC. *In vitro* antitumor activites was